



P.B.5818 - Patentlaan 2  
2280 HV Rijswijk (ZH)  
☎ +31 70 340 2040  
TX 31651 epo nl  
FAX +31 70 340 3016

**Europäisches  
Patentamt**

Zweigstelle  
in Den Haag  
Recherchen-  
abteilung

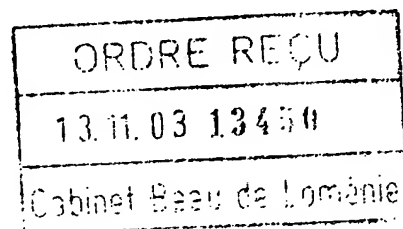
**European  
Patent Office**

Branch at  
The Hague  
Search  
division

**Office européen  
des brevets**

Département à  
La Haye  
Division de la  
recherche

Hubert, Philippe  
Cabinet Beau de Loménie  
158, rue de l'Université  
75340 Paris Cédex 07  
FRANCE



Datum/Date  
10.11.03

Zeichen/Ref./Réf. <b>3J331050/2/JHD</b>	Anmeldung Nr./Application No./Demande n°/Patent Nr./Patent No./Brevet n°. <b>03291540.7-2402-</b>
Anmelder/Applicant/Demandeur/Patentinhaber/Propriétaire/Titulaire <b>Nichirei Corporation</b>	

## COMMUNICATION

The European Patent Office herewith transmits as an enclosure the European search report for the above-mentioned European patent application.

If applicable, copies of the documents cited in the European search report are attached.

☒ Additional set(s) of copies of the documents cited in the European search report is (are) enclosed as well.

The following specifications given by the applicant have been approved by the Search Division:

☒ abstract

☒ title

☐ The abstract was modified by the Search Division and the definitive text is attached to this communication.

The following figure will be published together with the abstract: 1

## REFUND OF THE SEARCH FEE

If applicable under Article 10 Rules relating to fees, a separate communication from the Receiving Section on the refund of the search fee will be sent later.





DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
X	LIU QIANG ET AL: "Overlapping PCR for bidirectional PCR amplification of specific alleles: A rapid one-tube method for simultaneously differentiating homozygotes and heterozygotes" GENOME RESEARCH, vol. 7, no. 4, 1997, pages 389-398, XP002259043 ISSN: 1088-9051 * abstract *	1-4	C12Q1/68
X	WO 02 057487 A (AMERSHAM BIOSCIENCES UK LTD ;KNOTT TIM (GB); SCHWARZ TEREK (UG); S) 25 July 2002 (2002-07-25) * abstract * * page 5, line 15 - page 6, line 13 * * p. 16: seq. #3, #4, #20, #21, #25 * * claims; examples 6,7 *	1	
X	WO 01 94638 A (APPLERA CORP) 13 December 2001 (2001-12-13) * page 29, line 3 - line 20; claims; figures *	1-4	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	WO 01 49880 A (QIAGEN GMBH ;OELMUELLER UWE (DE); KORFHAGE CHRISTIAN (DE)) 12 July 2001 (2001-07-12) * the whole document *	1-4	C12Q
A	WO 99 24452 A (LIN KUEI YING ;ISIS PHARMACEUTICALS INC (US); MATTEUCCI MARK D (US) 20 May 1999 (1999-05-20) * page 2, line 17 - page 6, line 5 * * page 25, line 9 - line 18 * * page 58, line 17 - line 25 * * page 64, line 35 - page 66, line 7; table 1 *	1	
The present search report has been drawn up for all claims			
Place of search MUNICH		Date of completion of the search 24 October 2003	Examiner Luzzatto, E
CATEGORY OF CITED DOCUMENTS			
X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document		T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons & : member of the same patent family, corresponding document	

5

EPO FORM 1503 03.92 (P04C01)



DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
A	WO 98 45479 A (ALBANY MEDICAL COLLEGE) 15 October 1998 (1998-10-15) * claims *	1	
A	US 2003/039992 A1 (SCHUTT CLARENCE E ET AL) 27 February 2003 (2003-02-27) * the whole document *	1-5	
A	EP 1 138 785 A (CANON KK) 4 October 2001 (2001-10-04) * paragraph '0094!; claim 9; example 6 *		
T	DATABASE CA 'Online! CHEMICAL ABSTRACTS SERVICE, COLUMBUS, OHIO, US; KOIZUMI, TAKESHI ET AL: "Improving DNA amplification and hybridization efficiency by attaching compounds or oligonucleotide to primer" retrieved from STN Database accession no. 139:96316 XP002259044 * abstract * & JP 2003 199568 A (NICHIREI CORP., JAPAN) 15 July 2003 (2003-07-15)		
The present search report has been drawn up for all claims			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
Place of search MUNICH		Date of completion of the search 24 October 2003	Examiner Luzzatto, E
<p>CATEGORY OF CITED DOCUMENTS</p> <p>X : particularly relevant if taken alone Y : particularly relevant if combined with another document of the same category A : technological background O : non-written disclosure P : intermediate document</p> <p>T : theory or principle underlying the invention E : earlier patent document, but published on, or after the filing date D : document cited in the application L : document cited for other reasons ..... &amp; : member of the same patent family, corresponding document</p>			

5

EPO FORM 1503 03.82 (P04C01)

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 03 29 1540

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report. The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

24-10-2003

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 02057487	A	25-07-2002	CA	2432016 A1	25-07-2002
			EP	1352096 A2	15-10-2003
			WO	02057487 A2	25-07-2002
WO 0194638	A	13-12-2001	AU	6823501 A	17-12-2001
			CA	2412413 A1	13-12-2001
			WO	0194638 A2	13-12-2001
WO 0149880	A	12-07-2001	DE	19963857 A1	26-07-2001
			WO	0149880 A2	12-07-2001
			EP	1242621 A2	25-09-2002
WO 9924452	A	20-05-1999	US	6028183 A	22-02-2000
			US	6007992 A	28-12-1999
			AU	1292899 A	31-05-1999
			CA	2309340 A1	20-05-1999
			EP	1027364 A2	16-08-2000
			JP	2001522860 T	20-11-2001
			WO	9924452 A2	20-05-1999
			US	2003072712 A1	17-04-2003
			US	2003176677 A1	18-09-2003
			US	6414127 B1	02-07-2002
WO 9845479	A	15-10-1998	US	5994071 A	30-11-1999
			AU	6949498 A	30-10-1998
			EP	0975803 A1	02-02-2000
			WO	9845479 A1	15-10-1998
			US	2002076695 A1	20-06-2002
US 2003039992	A1	27-02-2003	WO	02061137 A2	08-08-2002
EP 1138785	A	04-10-2001	JP	2001275677 A	09-10-2001
			EP	1138785 A2	04-10-2001
			US	2003170716 A1	11-09-2003
			US	2002197607 A1	26-12-2002
JP 2003199568	A	15-07-2003	NONE		



This application is covered by the extended European search report pilot project at present running within the European Patent Office, applied to all European patent applications filed as first filing and searched on or after 01.07.03. Under this project the EPO issues together with the search report an opinion on whether the application and the invention to which it relates meet the requirements of the EPC. This non-binding opinion is issued free of charge as a service. This opinion may be used as the basis for an informed decision as to whether it is desired to pursue the application further or not.

For further details of this pilot project, the applicant's attention is directed to the Official Journal edition 5/2003. If any further immediate questions or comments arise the EPO Customer Services: +31-70-340 4500 or +49-89-2399 2828 can be contacted.

**The attached opinion reveals that the application or the invention to which it relates appear not to meet the requirements of the Convention** (see comments on enclosed Form 2906).

If the applicant wishes to continue with this application the examination fee must be paid. Where appropriate amendments can be filed to address the objections raised in the opinion, thus shortening the overall procedure. If no amendments are filed, the opinion will be re-issued as the first official communication under Article 96(2) and Rule 51(2) EPC.

If the examination fee has already been paid and the right to the communication under Article 96(1) EPC has been waived for this application, the first official communication under Article 96(2) and Rule 51(2) EPC will be issued promptly.



The examination is being carried out on the **following application documents**:

Text for the Contracting States:

AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HU IE IT LU MC NL PT RO SE SI SK TR LI

**Description, pages:**

1-17 as originally filed

**Claims, No.:**

1-5 as originally filed

**Drawings, sheets:**

1/2-2/2 as originally filed

The following document/s (D) is/are referred to in this communication; the numbering will be adhered to in the rest of the procedure:

- D1: LIU QIANG ET AL: 'Overlapping PCR for bidirectional PCR amplification of specific alleles: A rapid one-tube method for simultaneously differentiating homozygotes and heterozygotes' GENOME RESEARCH, vol. 7, no. 4, 1997, pages 389-398, XP002259043 ISSN: 1088-9051
- D2: WO 02 057487 A (AMERSHAM BIOSCIENCES UK LTD ;KNOTT TIM (GB); SCHWARZ TEREK (UG); S) 25 July 2002 (2002-07-25)
- D3: WO 01 94638 A (APPLERA CORP) 13 December 2001 (2001-12-13).
- D4: WO 99 24452 A (LIN KUEI YING ;ISIS PHARMACEUTICALS INC (US); MATTEUCCI MARK D (US) 20 May 1999 (1999-05-20)

1) Novelty (Art. 54 EPC)

1.1 Claims 1-3 lack novelty over D1.

D1 discloses (see abstract and p. 390, col. 1, l. 10-14) a PCR method comprising



the use of primers to which a GC tail has been added. These primers allow a high efficiency amplification and prevent the phenomenon of "megaprimering".

D1, therefore, anticipates the subject-matter of claims 1-3.

- 1.2 D2 (WO-A-02057487) (see the passages cited in the Search Report) discloses the addition of fluorescent molecules such as FAM and Cy3 to the 5' end of a primer in order to increase the efficiency of amplification.

D2, therefore, is novelty-destroying for the subject-matter of claims 1-3.

- 1.3 D3 (WO-A-0194638) describes the addition of a GC tail to a primer to increase the efficiency of an "asynchronous" PCR (p. 29, l. 3-30). It thus anticipates the subject-matter of claims 1-4.

- 1.4 No available document discloses a method for increasing the specificity of hybridisation wherein one of the compounds listed in claim 5 is added to the 5'-end of the hybridisation probe.

The subject-matter of claim 5 is thus novel.

## 2) Inventive step (Art. 56 EPC)

- 2.1 D4 is considered as the closest prior art with respect to the subject-matter of claim 5. It discloses an increase in the melting temperature of the probe-target duplex ( $T_m$ ) and hence in hybridisation specificity due to the addition of specific groups to the 5'-end of an oligonucleotide (see ex. 2 and table 1).

In view of D4, the technical problem underlying the application can be defined as that of providing an alternative method for increasing hybridisation specificity.

The solution proposed by claim 5 is to link one of the specific groups listed in the claim to the 5'-end of the probe. This feature represents also the difference between D4 and the claimed subject-matter.

Oligonucleotides labelled at their 5'-end with groups such as FAM or Cy3 are well known in the art (see e.g. D3, ex. 9-10). However, none of the available documents suggests using any of the groups listed in claim 5 as a means to increase  $T_m$  and thus hybridisation specificity.

An inventive step for the subject-matter of claim 5 should thus be acknowledged.



- 3) Claim 1 lacks clarity (Art. 84 EPC) because the term “amplification efficiency” is vague and lacks a sufficiently precise technical meaning. The precise definition of this expression should be introduced into the claims (see Guidelines, C-III, 4.2), for instance based on the definition given in the description (p. 16, l. 1-7).
- 4) Concluding remarks

Should the application enter the examination phase, the following would apply:

- a) The Applicant would be requested to file new claims which take account of the above comments.
- b) **The description must be brought into conformity with the new claims to be filed;** care should be taken during revision, especially of the introductory portion including any statements of problem or advantage, not to add subject-matter which extends beyond the content of the application as originally filed, Article 123(2) EPC. To allow a speedy check as to whether the requirements of Art. 123(2) EPC are met, **the passages of the application as originally filed which are deemed to support the amendments should be clearly indicated.**
- c) The amendments should be filed by way of replacement pages, avoiding unnecessary recasting of the description. The Applicant should also take account of the requirements of Rule 36(1) EPC. In particular, fair copies of the amendments should be filed in triplicate. If handwritten amendments are submitted, they should be clearly legible for the printer.
- d) In order to expedite the procedure, the Applicant is kindly asked to clearly point out where the amendments have been made, possibly by enclosing a copy of the original pages with the corrections in manuscript.
- e) To meet the requirements of Rule 27(1)(b) EPC, the documents D1-D4 would have to be identified in the description.





- f) The amendments would have to be filed in triplicate taking into account the requirements of R. 36(1) EPC. The passages in the application as originally filed which support the amendments should be clearly indicated (Art. 123(2) EPC). To expedite the procedure, the Applicant is kindly asked to clearly indicate where the amendments have been made, possibly by filing a copy of the original sheets with the hand-written amendments.
- g) The Applicant's attention is drawn to the requirements of R. 29(2) EPC which allows only one independent claim per category unless the exceptional situations listed in R. 29(2) (a-c) EPC apply. Should new claims be filed, care should be taken to comply with these requirements